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# Neural superposition and oscillations in the eye of the blowfly

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**Summary.** 1. Neural superposition in the eye of the blowfly *Calliphora erythrocephala* was investigated by stimulating single photoreceptors using corneal neutralization through water immersion. Responses in Large Monopolar Cells (LMCs) in the lamina were measured, while stimulating one or more of the six photoreceptors connected to the LMC. Responses to flashes of low light intensity on individual photoreceptors add approximately linearly at the LMC. Higher intensity light flashes produce a maximum LMC response to illumination of single photoreceptors which is about half the maximum response to simultaneous illumination of the six connecting photoreceptors. This observation indicates that a saturation can occur at a stage of synaptic transmission which precedes the change in the post-synaptic membrane potential.

2. Stimulation of single photoreceptors yields high frequency oscillations (about 200 Hz) in the LMC potential, much larger in amplitude than produced by simultaneous stimulation of the six photoreceptors connected to the LMC. It is discussed that these oscillations also arise from a mechanism that precedes the change in the post-synaptic membrane potential.

## Introduction

The fly has a neural superposition eye (Kirschfeld 1967) and the axons of eight separate photoreceptor cells converge in each cartridge of the lamina. Although the photoreceptors belong to different ommatidia, each one receives light from the same direction. This scheme was first suggested by Vigier (1907, as cited in Braitenberg and Strausfeld 1973), and confirmed optically by Kirschfeld (1967) and anatomically by Braitenberg (1967). Of these eight photoreceptor cells (R1–8), R1–6 have synapses onto second order neurons in the lamina, whereas R7 and R8 synapse outside the lamina (but see Shaw 1984). Apparently, the photoreceptor cells R1–R6 add (superpose) their responses at the second order lamina cells, which gives rise to the name neural superposition eye. Such an arrangement increases the amount of light collected from a given direction, i.e. seven facets collect light from one point in space instead of only one, with six facets contributing to neural superposition.

It has not been shown rigorously that responses from the six photoreceptors of the superposition projection add linearly in an LMC. Scholes (1969) investigated responses from the lamina, but the depolarizing responses are not from LMCs; a close examination of the methods and results of this study strongly suggests that the responses were extracellular lamina potentials and not axon terminal recordings of photoreceptor cells. Shaw (1984) has tested superposition in the axon terminals of photoreceptor cells but not in LMCs.

The aim of the present study is to test directly the theory of neural superposition by recordings from LMCs whilst using the microstimulation method as described by van Hateren (1986). Neural superposition was tested by stimulating one or all of the photoreceptor cells projecting to the LMC. Surprisingly, it was found that stimulation of a single photoreceptor cell yields fast oscillations (about 200 Hz) of the LMC potential.

*Abbreviation: LMC* large monopolar cell

## Methods

*Animals and preparation.* Experiments were performed on females of the blowfly *Calliphora erythrocephala* (wild type). The preparation was similar to that described in van Hateren (1986) and prior to measurement the flies were dark-adapted for 30–45 min.

*Electrophysiological recordings.* Conventional glass microelectrodes were used and filled with a mixture of 3 *M* KAc and 0.1 *M* KCl, typically having resistances of 150–200 MΩ. LMC recordings were identified by a characteristic transient hyperpolarization in response to a light flash. The name Large Monopolar Cell is used for a group of three lamina cells, L1, L2, and L3 with very similar response characteristics (Laughlin 1980). Since these are the largest cells in the lamina cartridges, a hyperpolarizing unit recorded in the lamina is likely to be a LMC. Most of the recordings in this study were obtained from the lamina part of the LMC, as evidenced by the axon terminal recordings of photoreceptor cells close to the LMC.

Extracellular recordings from the H1-neuron (Fig. 7) were made from the contralateral lobula plate using a tungsten microelectrode (see Mastebroek 1974); the preparation is described by de Ruyter van Steveninck (1986, p 107).

*Optical stimulation.* The method of optical stimulation was similar to van Hateren (1986). Briefly, light from a light guide inserted in a hole at the back of the head of the fly, propagates through the rhabdomeres which function as light guides, towards a water immersion microscope. The water immersion neutralizes the cornea (Franceschini 1975) so that the rhabdomeres become visible beneath the corneal lenses. The light stimulus is imaged onto the rhabdomeres which are observed along an optical plane where both the stimulus and rhabdomeres are visible. By using this method, all rhabdomeres can be stimulated individually under visual control. The stimulus consisted of LEDs (Siemens LD57C) having a spectral peak at 560 nm with a half-width of 25 nm.

*Stimulus generation and data acquisition.* Both stimulus generation and data acquisition were performed by a Data General Desktop 20 microcomputer (see van Hateren 1986). Tests showed that no pre-filtering was necessary prior to sampling the response with an A D-converter; responses were averaged on line and displayed on a graphics terminal. All data were stored on a Winchester disk for off-line analysis and also transferred to a DG Eclipse minicomputer for further processing.

The data acquisition for Fig. 7 (H1-neuron) was performed by a DG Nova microcomputer (see Zaagman 1977). Spike interval times were digitized with a resolution of 50 μs and stored on disk for off-line analysis.

## Results

The method of microstimulation (van Hateren 1986) allows individual stimulation of each photoreceptor within a restricted area of the retina. Recordings from LMCs involve usually six photoreceptor cells (R1–R6) which yield the largest LMC response to illumination (see Fig. 1). The photoreceptor cells are positioned in ommatidia as expected from the known anatomical projections from photoreceptor cells to LMCs (Braitenberg

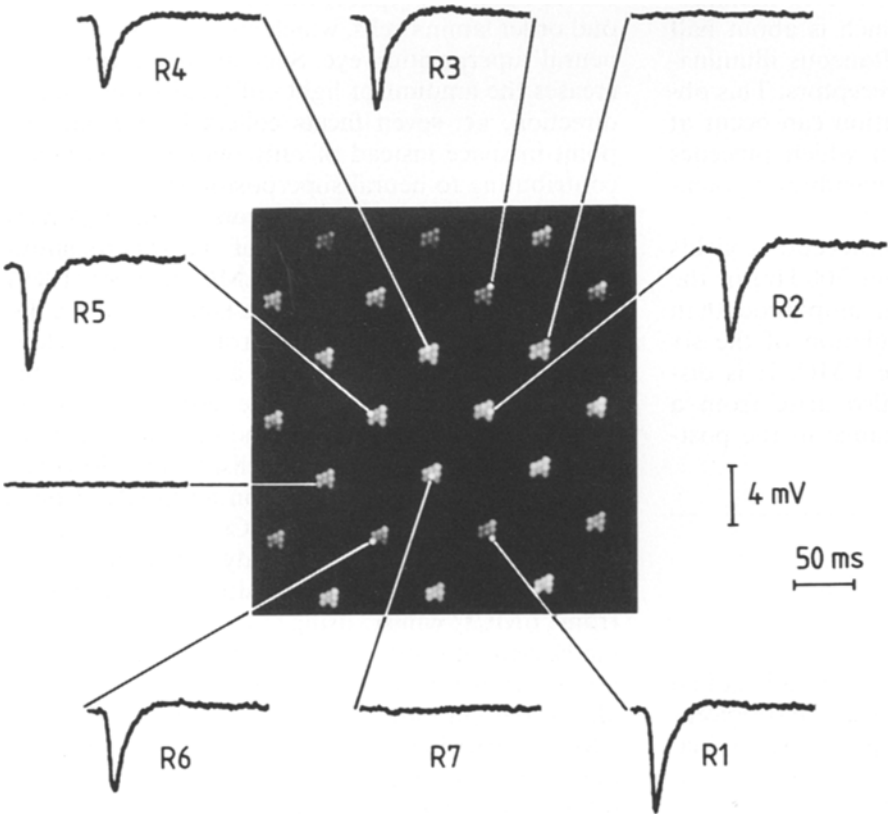


**Fig. 1.** Responses of LMCs to stimulation of various photoreceptors using 2 ms light flashes of low intensity (average of 32 stimuli). Light dots correspond to rhabdomeres, each group of seven belonging to one ommatidium. Dots within each group are separated by about 2 μm. The corneal lenses are neutralized by water immersion (see Franceschini 1975). The seven brightest dots are LEDs imaged onto rhabdomeres which would receive light from the same direction if the cornea were not neutralized (see van Hateren 1986 for method of stimulation). The corresponding photoreceptor cells project axons to a common cartridge in the lamina, where R1–6 synapse onto the LMCs. The responses to stimulation of R1–7 were measured in one LMC, the responses to stimulation of an R6 and an R3 in neighbouring neuro-ommatidium (unmarked traces) were measured in other LMCs, giving similar responses to illumination of R1–6 as illustrated

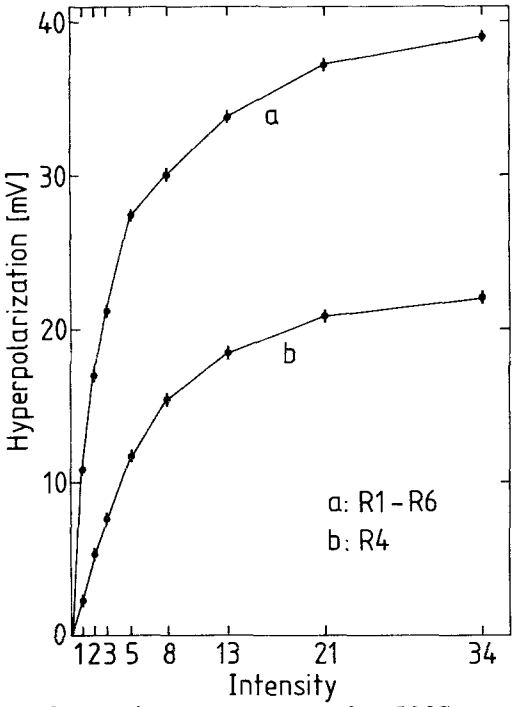


**Fig. 2.** Intensity-response curves of an LMC, recorded in the lamina neuropil, with illumination of all six neuro-ommatidial photoreceptor cells (*a*), and only one (R4, *b*). The abscissa (linear) shows the intensity of a 2 ms light flash (arbitrary units). The ordinate shows the peak hyperpolarization voltage of the response to each light flash intensity. Each data point is the average of 80 responses. Stimuli for each curve consisted of 80 presentations (alternating for a and b), where the order of stimulus intensity was 5, 21, 2, 34, 8, 3, 1, and 13

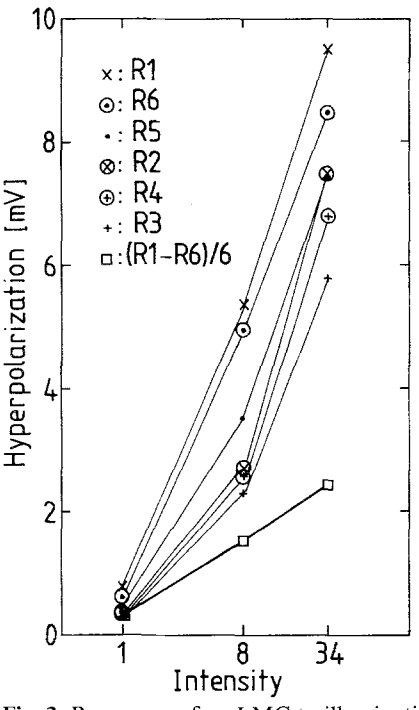


**Fig. 3.** Responses of an LMC to illumination of corresponding R1, R2, R3, R4, R5, and R6; and R1–6 simultaneously (squares = response reduced by one-sixth to enable comparison with the other responses). The abscissa (logarithmic) shows the intensity of a 2 ms light flash (arbitrary units), the ordinate the peak hyperpolarization voltage of the response. Average of 80 responses. Order of stimulus presentation: intensity 34, 8, and 1

1967). For convenience, R1–6 are referred to as the neuro-ommatidial photoreceptor cells (van Hateren 1986). Occasionally, one of the six photoreceptors yielded no or very little response; possibly, this was caused by damage to the axon from the microelectrode. On one occasion only a single photoreceptor responded (c.f. Shaw 1984), in a similar fashion to more conventional recordings. Often, illumination of the central R7–8 or other photoreceptor cells yielded responses also, either depolarization or hyperpolarization, but much smaller in magnitude compared to the neuro-ommatidial photoreceptor cells (see Fig. 1). A systematic investigation of these responses has not been made and this study will concentrate on the LMC response to illumination of the six neuro-ommatidial photoreceptor cells.

Figure 2 shows two intensity-response curves measured in a dark adapted LMC; in Fig. 2 (b) a single photoreceptor cell (R4) is illuminated and in Fig. 2 (a) all six neuro-ommatidial photoreceptor cells are illuminated. From Fig. 2 it can be seen that stimulation of a single receptor saturates the LMC response at a considerably lower amplitude compared to the maximum response produced by stimulation of six photoreceptors. For all cells tested, the maximum response with six cells stimulated was about twice the saturated response for one cell. At low light intensities, however, the response to illumination of six cells is approximately sixfold the response to illumination of a single cell, as expected by the theory of neural superposition. That is, the responses to illumination of individual photoreceptors add linearly when low amplitude.

Illumination of photoreceptors often yields responses in the corresponding LMC that differ between photoreceptors, as shown in Fig. 3. Part of response variability may be due to a difference in sensitivity of the photoreceptor cells and in efficiency of synapses from photoreceptor cells to LMC. However, another source of variability is the optical stimulation, and although the LEDs were imaged onto the photoreceptors as well as possible, variation in illumination cannot be excluded. Moreover, almost all flies made small retinal movement despite excluding animals with substantial eye movement.

Figure 3 also shows the LMC reponse to simul-

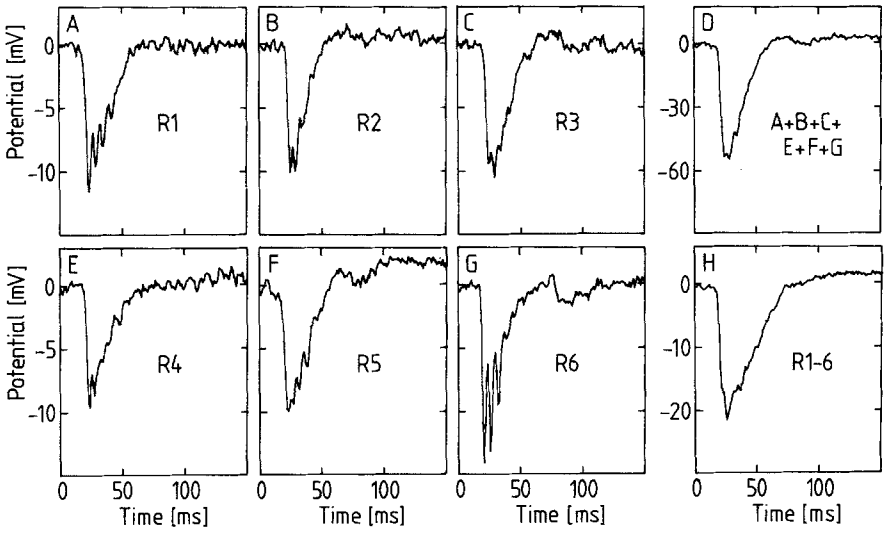


**Fig. 4A–H.** Responses of an LMC to 2 ms light flashes. **A, B, C, E, F,** and **G** show responses to single flashes (not averaged). Note the pronounced high frequency oscillations in several of these responses. In **D** the responses of **A, B, C, E, F,** and **G** were added, to compare with the response to a 2 ms light flash on R1–6 together **(H)**

taneous illumination of R1–6. The magnitude of this response was reduced by a factor of six for comparison with the response to illumination of single photoreceptors. For low intensity flashes (small responses) all experiments showed no significant deviation from the neural superposition scheme but at higher intensities, the LMC response to illumination of six cells was considerably smaller than expected – corresponding to the effect on saturated response levels as shown in Fig. 2.

Some of the properties of neural superposition indicate a more complex process than the simple addition of photoreceptor cell responses after processing by a sign inverting synapse. In Fig. 4 responses (not averaged) are shown to light flashes on single photoreceptors, illustrating superposition of these responses (Fig. 4D), and the response to a light flash on all R1–6 photoreceptors (Fig. 4H). It can be seen in Fig. 4 that a light flash on single photoreceptors often causes fast oscillations in the LMC response. For illumination of individual photoreceptors these oscillations vary in modulation depth and have a slightly different amplitude and frequency between illumination of different cells (see Discussion). Simultaneous illumination of all R1–6 cells may yield oscillations (Laughlin and Hardie 1978) but these are usually much less pronounced (Fig. 4H). In Fig. 4D the responses to illumination of R1–R6 were added and comparison of Fig. 4D with Fig. 4H shows that the response of the latter has a smaller peak (as in Fig. 2) and a longer time course. Such an effect is not surprising, because a longer response duration can be observed in the photoreceptor cells already, especially in the axon terminals (van Hateren 1986). This phenomenon is, at least in part, caused

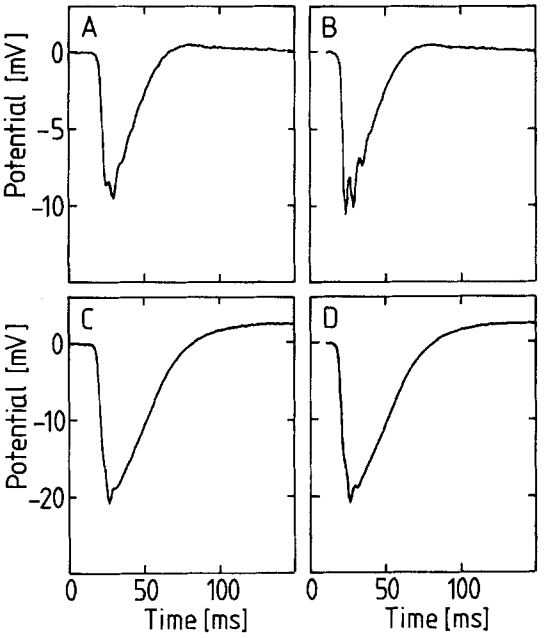


**Fig. 5. A** Averaged response of the LMC shown in Fig. 4 to 2 ms flashes on R1, c.f. Fig. 4A. **B** Same as **A**, but averaged after triggering at the front edge of the response. **C** Averaged response to 2 ms flashes on R1–6 together c.f. Fig. 4H. **D** Same as **C**, but averaged after triggering at the front edge of the response (see text for details). Average of 80 responses

by the gap junctions that electrically couple the neuro-ommatidial photoreceptor cells, but other mechanisms, such as feedback through voltage-sensitive channels in the axon terminals, or feedback from higher order neurons can not be ruled out at present (van Hateren 1986).

Figure 5 shows examples of averaged responses taken from the same experiment as Fig. 4. In Fig. 5A R1 is illuminated alone, whilst in Fig. 5C

all six receptors (R1–6) are simultaneously illuminated. These averaged responses do not show the oscillations seen clearly in individual records, e.g. Fig. 4A. In this case the absence of oscillations is due in part to variation in the temporal pattern in individual responses: averaging thus degrades the oscillations. To show the effect of averaging, the responses that produced Fig. 5A were averaged, not in relation to the stimulus, but relative to the time at which each response reached half of the maximum amplitude. The result of this procedure is shown in Fig. 5B and clearly reveals the oscillations. Applying the same procedure to responses to illumination of R1–6 (Fig. 5C) shows that the oscillations are rather indistinct (Fig. 5D); a similar difference exists between the response to single flashes as seen in Fig. 4A and H.

## Discussion

The experiments presented in Figs. 2 and 3 were designed to test directly the theory of neural superposition and indeed, low amplitude responses from the six photoreceptors of the superposition projection add linearly in the LMC. Large amplitude responses do not add linearly due to saturation. These results demonstrate that microstimulation is a powerful method for investigating the interactions within neural circuits (see also van Hateren 1986).

The results in Fig. 2 show that LMCs saturate at different levels, depending on the number of photoreceptors illuminated. Response curves corresponding to illumination of two up to five cells form a continuous series (not illustrated) between the curves of one and six cell illumination. The saturated response in an LMC may originate from post-synaptic influences, as set by the reversal potential for the hyperpolarizing response for example. However, there would then be no difference in amplitude of the saturated response between the number of illuminated photoreceptor cells. The difference in the saturated response amplitude therefore suggests that at least part of the saturation is not situated in the post-synaptic membrane but is localized to the receptor terminals and the processes of synaptic transmission. Measurement of responses in the axon terminals of photoreceptor cells (van Hateren 1986) does not indicate a response saturation as strong as illustrated in Fig. 2. Thus saturation during synaptic transmission seems likely, e.g. a saturation of calcium influx, transmitter release, or the postsynaptic receptors for the transmitter.

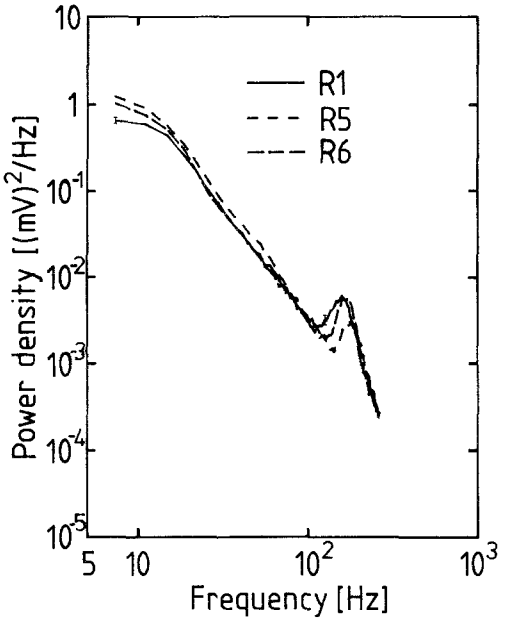


**Fig. 6.** Power spectra of the responses of the LMC shown in Fig. 4, to 2 ms flashes on single photoreceptor cells (R1, R5, and R6). Average power spectrum of 80 responses in each case. In the R1 curve, error bars at the lowest frequency and at 125 Hz show the standard deviation of the mean. Errors at other frequencies and in other curves are comparable

### *Oscillations*

Illumination of single photoreceptors produces a clear oscillation in the response of an LMC (Figs. 4 and 5). This is true even for the response to single photons and favourable LMC recordings show large bumps (5–10 mV) with marked oscillations. Again, these oscillations may have a post- or presynaptic origin. For example, a post-synaptic origin may be due to a membrane with voltage-sensitive channels. From both theory and experimental evidence (Sabah and Liebovic 1969; Lewis and Hudspeth 1983), membranes with voltage-sensitive channels can display oscillatory behaviour without giving rise to full size action potentials. A post-synaptic voltage dependent mechanism is not very likely in the present case, however, for two reasons. First, injecting current into the LMC generally does not cause oscillations (Dr. S.B. Laughlin, pers. comm.). Secondly, oscillations resulting from the illumination of different photoreceptor cells appear to be independent of each other. This effect is illustrated in Fig. 6, where the averaged power spectra of the responses to illumination of single photoreceptors are shown (R1, R5, and R6). The oscillations appear as a pronounced peak in the spectrum at a frequency of about 200 Hz. These high frequency peaks have amplitude, width and peak frequency which differ significantly between

photoreceptors, as shown by error analysis. These peaks would not vary if the oscillations originated from a common voltage-sensitive post-synaptic mechanism, at least if it is assumed that the lamina part of the LMC is approximately iso-potential.

The oscillations must arise, therefore, from a process restricted to each photoreceptor cell and its synapse. A pre-synaptic origin might be an oscillation in the pre-synaptic voltage which in turn modulates $Ca^{2+}$ influx (through voltage sensitive $Ca^{2+}$ channels) and effects transmitter release. Such oscillations (at about 200 Hz) are observed sometimes in the axon terminals, e.g. the notch discussed by Shaw (1981, 1984) and van Hateren (unpublished), but only occur with high intensity light flashes. In the present study, light intensities already yielding clear oscillations in an LMC, were not observed to yield comparable oscillations in the pre-synaptic potential.

Another possibility for the origin of oscillations is that the transmitter release oscillates without being mediated by the pre-synaptic potential. One source of modulation of transmitter release could be due to fluctuation in $Ca^{2+}$ influx but this hypothesis causes two problems. In the first place, oscillation in $Ca^{2+}$ influx is also an oscillation in $Ca^{2+}$ current and must therefore produce an oscillation in the membrane potential, which is not observed. Of course, in order to stop the pre-synaptic potential regenerating, the potential generated by $Ca^{2+}$ influx must either be opposed by antagonistic conductances (such as potassium or chloride), or be negligible. The $Ca^{2+}$ influx potential may well be negligible, because the input resistance of the cell as seen from the axon terminal is quite low (similar to the input resistance in the cell body, i.e. 30 MΩ, see van Hateren 1986). This would explain why the 200 Hz oscillations in the pre-synaptic membrane potential are observed clearly only with flashes of high light intensities i.e. producing larger $Ca^{2+}$ current.

The second problem arising from the hypothesis of an oscillation in $Ca^{2+}$ influx is that $Ca^{2+}$ influx in synapses is due generally to $Ca^{2+}$ channels being voltage sensitive. Pre-synaptic voltage fluctuations would be absent, then, only if the $Ca^{2+}$ influx was partially modulated chemically perhaps by neuro-transmitters from neurones synapsing onto the axon terminals (L2, amacrines). A direct influence of neuro-transmitters on $Ca^{2+}$ channels has been reported recently (Reuter 1985; for review Reuter 1983) but it remains difficult to understand how such an influence causes these fast oscillations.

In principle, the oscillations may not be significant to visual processing in the fly and may possibly be just an epiphenomenon, related to powerful feedback mechanisms that eventually determine the LMC response. Laughlin et al. (1987) demonstrate that the impulse response of an LMC is close to the sum of two exponentials, possibly arising from combined amplification and delayed antagonism. Oscillations may be a by-product of the processes which sharpen the impulse response of the LMC. This view is favoured by the observation that oscillations are usually less pronounced when illuminating six cells (the natural stimulus) compared to illumination of a single cell. Nevertheless, it is possible that oscillations play a more important role in the visual system, as damped oscillations of a similar frequency can be observed throughout the visual system, e.g. in field potentials recorded in the medulla or lobula (Burkhardt 1954) or in the H1-neuron. In the latter case, an example of an H1 neuron is shown in Fig. 7, demonstrating oscillations of about 200 Hz in the spike firing rate occurring at the off-transient of a light flash. However, these oscillations are usually elicited only by broad field stimuli, stimulating many photoreceptors and causing local oscillations at photoreceptor synapses. These oscillations are slightly out of phase and tend to cancel when added initially in the LMC and subsequently in the H1-neuron. The oscillations seen in field potentials and those shown in Fig. 7, could be related to the oscillations in LMCs only if broad field illumination brings the local oscillations into phase again. This could be done by coupling through field potentials or wide-field neurons such as amacrine cells in the lamina (see Shaw 1984). Such a hypothesis is certainly able to be tested experimentally.

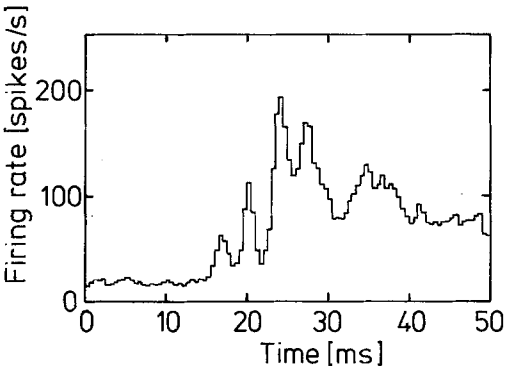


**Fig. 7.** Oscillations in the spike firing rate measured in the H1-neuron of the blowfly. The stimulus consisted of an LED placed close to the eye, giving 500 ms pulses of light with a frequency of 1 Hz. The post-stimulus time histogram is a section of the response immediately after light off; binwidth 0.5 ms, average of the responses to 5760 stimulus presentations

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## References

Braitenberg V (1967) Patterns of projection in the visual system of the fly. I. Retina-lamina projections. Exp Brain Res 3:271–298

Braitenberg V, Strausfeld NJ (1973) Principles of the mosaic organization in the visual system's neuropil of *Musca domestica* L. In: Jung R (ed) Central processing of visual information (Handbook of sensory physiology, vol VII/3A). Springer, Berlin Heidelberg New York, pp 631–660

Burkhardt D (1954) Rhythmische Erregungen in den optischen Zentren von *Calliphora erythrocephala*. Z Vergl Physiol 36:595–630

Franceschini N (1975) Sampling of the visual environment by the compound eye of the fly: Fundamentals and applications. In: Snyder AW, Menzel R (eds) Photoreceptor optics. Springer, Berlin Heidelberg New York, pp 98–125

Hateren JH van (1986) Electrical coupling of neuro-ommatidial photoreceptor cells in the blowfly. J Comp Physiol A 158:795–811

Kirschfeld K (1967) Die Projektion der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von *Musca*. Exp Brain Res 3:248–270

Laughlin SB (1980) Neural principles in the visual system. In: Autrum H (ed) Handbook of sensory physiology, vol VII/6B. Springer, Berlin Heidelberg New York, pp 133–280

Laughlin SB, Hardie RC (1978) Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. J Comp Physiol 128:319–340

Laughlin SB, Blakeslee B, Howard J (1987) Synaptic limitations to contrast coding in the retina of the blowfly *Calliphora*. (in press)

Lewis RS, Hudspeth AJ (1983) Voltage- and ion-dependent conductances in solitary vertebrate hair cells. Nature 304:538–541

Lillywhite PG, Dvorak DR (1981) Responses to single photons in a fly optomotor neurone. Vision Res 21:279–290

Mastebroek HAK (1974) Stochastic structure of neural activity in the visual system of the blowfly. Thesis, Groningen University

Matic T (1983) Electrical inhibition in the retina of the butterfly *Papilio*. J Comp Physiol 152:169–182

Reichardt W (1969) Transduction of single quantum effects. (Evidence from behavioral experiments on the fly *Musca*). In: Reichardt W (ed) Processing of optical data by organisms and by machines. Academic Press, London, pp 176–186

Reuter H (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature 301:569–574

Reuter H (1985) A variety of calcium channels. Nature 316: 391

Ruyter van Steveninck RR de (1986) Real-time performance of a movement-sensitive neuron in the blowfly visual system. Thesis, Groningen University

Sabah NH, Liebovic KN (1969) Subthreshold oscillatory responses of the Hodgkin-Huxley cable model for the squid giant axon. Biophys J 9:1206–1222

Scholes J (1969) The electrical responses of the retinal receptors and the lamina in the visual system of the fly *Musca*. Kybernetik 6:149–162

Shaw SR (1975) Retinal resistance barriers and electrical lateral inhibition. Nature 255:480–483

Shaw SR (1981) Anatomy and physiology of identified nonspiking cells in the photoreceptor-lamina complex of the compound eye of insects, especially Diptera. In: Roberts A, Bush BMH (eds) Neurons without impulses. Cambridge University Press, Cambridge, pp 61–116

Shaw SR (1984) Early visual processing in insects. J Exp Biol 112:225–251

Zaagman WH (1977) Some characteristics of the neural activity of directionally selective movement detectors in the visual system of the blowfly. Thesis, Groningen University